

Collective oscillation period of inter-coupled biological negative cyclic feedback oscillators

Yongqiang Wang, *Senior Member, IEEE*, Yutaka Hori, *Member, IEEE*, Shinji Hara, *Fellow, IEEE*, Francis J. Doyle III, *Fellow, IEEE*

Abstract

A number of biological rhythms originate from networks comprised of multiple cellular oscillators. But analytical results are still lacking on the collective oscillation period of inter-coupled gene regulatory oscillators, which, as has been reported, may be different from that of an autonomous oscillator. Based on cyclic feedback oscillators, we analyze the collective oscillation pattern of coupled cellular oscillators. First we give a condition under which the oscillator network exhibits oscillatory and synchronized behavior. Then we estimate the collective oscillation period based on a novel multivariable harmonic balance technique. Analytical results are derived in terms of biochemical parameters, thus giving insight into the basic mechanism of biological oscillation and providing guidance in synthetic biology design.

I. INTRODUCTION

Diverse biological rhythms are generated by multiple cellular oscillators that operate synchronously. In systems ranging from circadian rhythms to segmentation clocks, it remains a challenge to understand how collective oscillation patterns (e.g., period, amplitude) arise from autonomous cellular oscillations. As has been reported in the literature, there can be significant differences between collective oscillation patterns and cell autonomous oscillation patterns. The differences are embodied not only in the oscillation amplitude [2], but also in the oscillation period [3], [4].

A special case of the work (inter-coupled Goodwin oscillators) was published in IEEE CDC 2012 [1]. The work was supported in part by NIH (GM096873), ICB (W911NF-09-0001, W911NF-09-D-0001-0027) from U.S. ARO, JSPS (23-9203), and GASR (A) (21246067). Y. Wang, F. Doyle are with Department of Chemical Engineering, University of California, Santa Barbara, 93106 USA. E-mail: wyqthu@gmail.com, frank.doyle@icb.ucsb.edu. Y. Hori and S. Hara are with Department of Information Physics and Computing, The University of Tokyo, Tokyo 113-8656 Japan. E-mail: {Yutaka_hori, Shinji_hara}@ipc.i.u-tokyo.ac.jp

Negative feedback is at the core of many biological oscillators [5]. One widely studied feedback mechanism in biological oscillators is the cyclic feedback of a sequence of biochemical reactions, where each reaction product activates the subsequent reaction while the end-product inhibits the first reaction [6]. This type of structure is not only used to formulate enzymatic control processes, but is also found in metabolic and cellular signaling pathways [7]. An advantage of such an oscillator is that it allows for an analytical understanding of basic dynamical mechanisms. For example, the oscillation conditions of a single negative cyclic feedback oscillator were obtained in [8], [9], [10], [11]. The synchronization condition for a network of such oscillators was reported in [12]. The oscillation patterns of a single such oscillator were also obtained in [13], [14]. This is an important step toward understanding the period determination in biochemical oscillators. However, it remains a challenge to determine the periods in biological rhythms generated by **multiple** cellular oscillators. Recently, using the phenomenological phase model, the authors in [15] proved that if intercellular coupling is weak, the collective period is identical to the autonomous period. However, since the phase model contains no direct biological mechanism of cellular clocks, its utility is limited when it comes to checking scientific hypotheses.

This paper analyzes the collective period of inter-coupled negative cyclic feedback oscillators. The key idea is to decompose the whole system into scalar subsystems and then use a multivariable harmonic balance technique. The multivariable harmonic balance technique has been adopted in [16] to study central pattern generators. However, since [16] assumes that the average value of oscillation is zero, its results are not applicable to gene regulatory oscillators. This is because, firstly, variables in gene regulatory oscillators denote concentrations of chemical reactants and cannot be negative, thus do not have zero average values; secondly, as indicated in [13], the zero-average-value assumption is only true when the nonlinearity is odd, which is not the case here. In this paper, we developed a new multivariable harmonic balance technique that is applicable to gene regulatory oscillators. Due to the removal of the zero-average-value assumption, the harmonic balance equations become very difficult to solve. Here we are interested in the collective period, so we circumvent the problem by concentrating on synchronized oscillations. To this end, we also give an oscillation/synchronization condition. It is worth noting that the oscillation condition for coupled oscillators is different from that of a single oscillator, as diffusive coupling may lead to oscillations in an otherwise stable system [17].

It is worth noting that although our previous results [18] gave an estimation for the collective

oscillation period of a **special** type of biological cyclic feedback oscillators connected in a **restrictive all-to-all** manner, systematic studies are still lacking for the collective oscillation analysis of **general** cyclic feedback oscillators coupled with **general** intercellular interactions. This paper is an endeavor in this direction. We give a method to decompose the network dynamics under a general coupling structure, which is the key to derive the results. This paper builds on the results in [18] in a number of important ways: 1) the single oscillator model is more general; 2) distributed delays can be accommodated, which is more practical [19] than the discrete time lag; 3) intercellular coupling is diffusive rather than mutual repressive, and the interaction structure is more general than the all-to-all structure in [18]; 4) a synchronization condition is given, which is not discussed in [18]; 5) a framework is developed to study the stability of oscillations at the estimated frequency.

II. MODEL DESCRIPTION AND DECOMPOSITION

A. The model of a single oscillator

We first consider the dynamics of a **single** negative cyclic feedback oscillator [20]:

$$\begin{cases} d[P_1]/dT = \rho_0/(1 + [P_M/K_0]^p) - k_1[P_1] \\ d[P_m]/dT = \rho_{m-1}[P_{m-1}] - k_m[P_m], \quad m = 2, 3, \dots, M \end{cases} \quad (1)$$

Here $[P_m] \in \mathbb{R}^1$ is the concentration of the product P_m (e.g., mRNA, protein, metabolite) in the m th reaction ($1 \leq m \leq M$); ρ_m ($0 \leq m \leq M-1$) are the rates of synthesis; k_m ($1 \leq m \leq M$) are degradation rates; $1/K_0$ is the binding constant of the end product to the transcription factor; and p is the Hill coefficient, which describes the cooperativity of end product repression.

Remark 1: The cyclic feedback in (1) has been used to model the oscillations in various enzymatic control processes [20] and metabolic control processes [10], [21].

Remark 2: Distributed delays involved in transcription, translation, and end product inhibition can also be incorporated in the negative cyclic feedback in (1). According to the ‘linear chain trick’, their cumulative effects simply amount to increasing the length of the feedback loop and the increased length is proportional to the average magnitude of the distributed delay [19].

The negative cyclic feedback oscillator in (1) can be transformed into a dimension-less form

$$\begin{cases} dx_1/dt = f(x_M) - b_1x_1 \\ dx_m/dt = x_{m-1} - b_mx_m, \quad m = 2, 3, \dots, M \end{cases}, \quad f(x) = \frac{1}{1 + x^p} \quad (2)$$

by $\varsigma = \sqrt[M]{(\prod_{i=0}^{M-1} \rho_i)/K_0}$, $\nu_M = \frac{1}{K_0}$, $\nu_{j-1} = \frac{\rho_{j-1}\nu_i}{\varsigma}$, $x_m = \nu_m[P_m]$, $t = \varsigma T$, and $b_i = \frac{\rho_i}{\varsigma}$ [20].

Transformation from (2) to (1) reduces parameters and thus facilitates an analytical treatment.

B. The model of interconnected oscillators

Next we consider a network of N oscillators with each oscillator described by (2) (cf. Fig. 1). Following [22], we assume that one synchronizing factor (the k th reaction product x_k ($2 \leq k \leq M$)) connects the oscillators by diffusion. Then the network dynamics is given by

$$\begin{cases} dx_{1,i}/dt = f(x_{M,i}) - b_1 x_{1,i} \\ dx_{m,i}/dt = x_{m-1,i} - b_m x_{m,i}, \quad 2 \leq m \leq M, m \neq k \\ dx_{k,i}/dt = x_{k-1,i} - b_k x_{k,i} - \sum_{j=1, j \neq i}^N a_{i,j} (x_{k,i} - x_{k,j}) \end{cases} \quad (3)$$

where $i = 1, 2, \dots, N$ denotes the index of oscillator i , and $a_{i,j} \geq 0$ denotes the coupling strength between oscillators i and j . If $a_{i,j} = 0$, then there is no interaction between oscillators i and j .

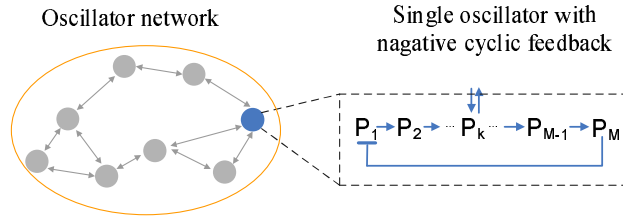


Fig. 1. A network of N oscillators. In each oscillator, P_i ($1 \leq i \leq M-1$) activates P_{i+1} , P_M inhibits the production of P_1 .

Assumption 1: We assume $a_{i,j} = a_{j,i}$, which follows from the characteristics of diffusion processes. We also assume connected interaction, i.e., there is a multi-hop path (i.e., a sequence with nonzero $a_{i,m_1}, a_{m_1,m_2}, \dots, a_{m_{p-1},m_p}, a_{m_p,j}$) from each node i to every other node j .

Remark 3: Assumption 1 is quite general. The commonly used all-to-all interaction [2], nearest neighbor interaction [4], and grid interaction [23] all satisfy Assumption 1.

For convenience in analysis, we can recast (3) in the following matrix form:

$$\begin{cases} dX_1/dt = \vec{f}(X_M) - b_1 X_1 \\ dX_m/dt = X_{m-1} - b_m X_m, \quad 2 \leq m \leq M, m \neq k, \\ dX_k/dt = X_{k-1} - b_k X_k - AX_k \end{cases} \quad X_m = \begin{bmatrix} x_{m,1} \\ x_{m,2} \\ \vdots \\ x_{m,N} \end{bmatrix} \in \mathbb{R}^{N \times 1} \quad (4)$$

$$\vec{f}(X_M) = \begin{bmatrix} f(x_{M,1}) \\ f(x_{M,2}) \\ \vdots \\ f(x_{M,N}) \end{bmatrix} \in \mathbb{R}^{N \times 1}, A = \begin{bmatrix} \sum_{j \neq 1} a_{1,j} & -a_{1,2} & \dots & -a_{1,N} \\ -a_{2,1} & \sum_{j \neq 2} a_{2,j} & \dots & -a_{2,N} \\ \vdots & \ddots & \ddots & \vdots \\ -a_{N,1} & \dots & -a_{N,N-1} & \sum_{j \neq N} a_{N,j} \end{bmatrix} \in \mathbb{R}^{N \times N} \quad (5)$$

Since A is symmetric and has zero row-sums, it can be diagonalized by some matrix P :

$$A = P\Upsilon P^{-1}, \quad \Upsilon = \text{diag}(v_1, v_2, \dots, v_N) \in \mathbb{R}^{N \times N} \quad (6)$$

where $0 = v_1 < v_2 \leq \dots \leq v_N$. The eigenvalue 0 is associated with eigenvector $[1 \ 1 \ \dots \ 1]^T$ [24]. v_2 measures the connectivity of interaction. It is positive when interaction is connected, and is greater when the interaction is stronger [24].

C. Decomposition of the interconnected oscillator network model

We are interested in the condition for oscillatory dynamics of the oscillator network in (4), so it is necessary to analyze its equilibrium. Next we show that (4) has one unique equilibrium.

At the equilibrium point, we have $dX_m^*/dt = 0$, $m = \{1, 2, \dots, M\}$, which yields

$$g(x_{M,i}^*) \triangleq f(x_{M,i}^*) - \prod_{m=1}^M b_m x_{M,i}^* = \prod_{m=1, m \neq k} b_m \sum_{j \neq i} a_{i,j} (x_{M,i}^* - x_{M,j}^*) \quad (7)$$

Since the interaction is bi-directional, i.e., $a_{i,j} = a_{j,i}$, it follows

$$\sum_{i=1}^N g(x_{M,i}^*) = \sum_{i=1}^N \sum_{j \neq i} a_{i,j} (x_{M,i}^* - x_{M,j}^*) = 0 \quad (8)$$

Next, we prove that (9) holds by proving that both $\max_i \{g(x_{M,i}^*)\}$ and $\min_i \{g(x_{M,i}^*)\}$ are zero:

$$g(x_{M,1}^*) = g(x_{M,2}^*) = \dots = g(x_{M,N}^*) = 0 \quad (9)$$

Suppose to the contrary that (9) does not hold, then $\max_i \{g(x_{M,i}^*)\} > 0$ since $\sum_{i=1}^N g(x_{M,i}^*) = 0$ holds according to (8). Represent the index of the largest $g(x_{M,i}^*)$ among all $1 \leq i \leq N$ as q . Then $x_{M,q}^*$ should be the smallest among $x_{M,1}^*, x_{M,2}^*, \dots, x_{M,N}^*$ because $f(\bullet)$ and hence $g(\bullet)$ is a decreasing function (cf. definition in (7)). Therefore, the rightmost hand side of (7) should be non-positive, and hence $g(x_{M,q}^*) < 0$. This contradicts the fact that $g(x_{M,q}^*)$ is the largest among $g(x_{M,i}^*)$ and is positive (due to the constraint in (8)). Hence $\max_i \{g(x_{M,i}^*)\} = 0$ holds. Similarly, we can prove $\min_i \{g(x_{M,i}^*)\} = 0$. Therefore, we have (9), which further leads to

$$f(x_{M,i}^*) = Bx_{M,i}^*, \quad i = 1, 2, \dots, N, \quad B \triangleq \prod_{m=1}^M b_m \quad (10)$$

Since $f(x)$ is monotonic decreasing for $x \geq 0$, the solution to (10) is unique and it satisfies

$$x_{M,1}^* = x_{M,2}^* = \dots = x_{M,N}^* = x_0 > 0, \quad f(x_0) = Bx_0 \quad (11)$$

Therefore the solution to (7) is unique, thus the equilibrium point is unique.

An oscillatory solution of (4) needs unstable dynamics near the equilibrium. To check the dynamics of (4) near the equilibrium, we linearize the nonlinear item $\vec{f}(X_M)$ in (4) around X_M^*

$$\vec{f}(X_M - X_M^*) = -\sigma(X_M - X_M^*), \quad X_M^* = [x_0 \ x_0 \ \dots \ x_0]^T, \quad \sigma = \frac{px_0^{p-1}}{(1+x_0^p)^2} = px_0^{p+1}B^2 \quad (12)$$

Then the overall dynamics of (4) can be represented in the frequency domain, as in Fig. 2, where

$$H(s) = ((sI + b_k I + A) \prod_{m=1, m \neq k}^M (sI + b_m I))^{-1} = \frac{(sI + b_k I + A)^{-1}}{\prod_{m=1, m \neq k}^M (s + b_m)} \quad (13)$$

and the matrix $L \in \mathbb{R}^{N \times N}$ denotes the influence of the nonlinear term after linearization.

For a general matrix L , it is difficult to give an analytical treatment of the dynamics in Fig. 2. Fortunately, under the matrix formulation in (4), we can diagonalize the system and reduce it to multiple scalar subsystems. This is the key to derive the analytical results in this paper.

Using (4) and (12), we can get $L = \sigma I \in \mathbb{R}^{N \times N}$, and hence the overall dynamics in Fig. 2:

$$G(s) = (I + \sigma H(s))^{-1} H(s) \quad (14)$$

Substituting (6) into (13), we have

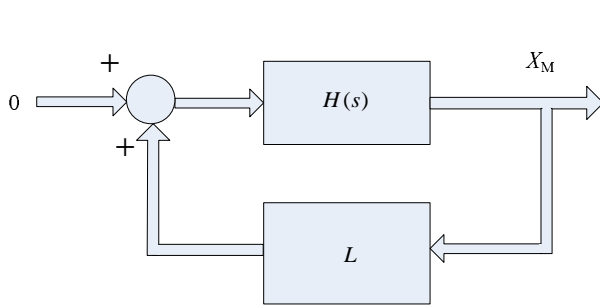


Fig. 2. Schematic diagram of the frequency domain formulation ($L = \sigma I$).

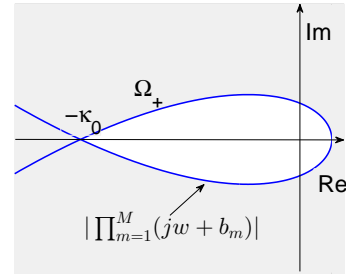


Fig. 3. Schematic diagram of unstable region (Ω_+). $\kappa_0 = \prod_{m=1}^M \sqrt{\mu^2 + b_m^2}$.

$$H(s) = P\Lambda P^{-1}, \quad \Lambda = \text{diag}(\lambda_1(s), \lambda_2(s), \dots, \lambda_N(s)) \quad (15)$$

where $\lambda_j(s) = \frac{1}{(\prod_{m=1, m \neq k}^M (s + b_m))(s + b_k + v_j)}$ for $j = 1, 2, \dots, N$. Substituting (15) into (14) yields

$$G(s) = P(I + \sigma\Lambda)^{-1}\Lambda P^{-1} = P\Delta P^{-1}, \quad \Delta = \text{diag}(\delta_1(s), \delta_2(s), \dots, \delta_N(s)) \quad (16)$$

$$\delta_j(s) = \frac{\lambda_j(s)}{1 + \sigma \lambda_j(s)} = \frac{1}{(\prod_{m=1, m \neq k}^M (s + b_m))(s + b_k + v_j) + \sigma}, \quad j = 1, 2, \dots, N \quad (17)$$

So far, we have decomposed the network dynamics into multiple scalar subsystems, which, as will be shown later, greatly facilitates an analytical treatment of the network dynamics.

III. OSCILLATION/SYNCHRONIZATION CONDITION

A. Theoretical analysis of the oscillation/synchronization condition

To study the collective period, we need to guarantee that the X_m in (4) oscillate, and furthermore, oscillate in synchrony. We consider the Y-oscillation, which is defined below [16]:

Definition 1: A system $\dot{x} = f(x)$ with $x(t) \in \mathcal{R}^m$ is Y-oscillatory if each solution is bounded and there exists a state x_i such that $\lim_{t \rightarrow +\infty} x_i(t) < \overline{\lim}_{t \rightarrow +\infty} x_i(t)$ for almost all initial states $x(0)$.

To prove that (4) is Y-oscillatory, we introduce Lemma 1:

Lemma 1: [25] System (4) is Y-oscillatory if all conditions (a), (b), and (c) hold:

- (a) It only has isolated equilibria;
- (b) $\left\{ X(t) \triangleq [X_1^T(t), X_2^T(t), \dots, X_M^T(t)]^T | t \geq 0 \right\}$ is bounded;
- (c) The Jacobian matrices at equilibria have at least one unstable eigenvalue.

The result follows from these considerations: To get Y oscillations, we need to guarantee that 1) the linearized systems near the equilibrium points do not converge to constant values; 2) the solutions are bounded; and, 3) there exists a homeomorphism between solutions of the nonlinear system and its linearization. All of these can be obtained following Theorem 1 in [25] and the discussion below its proof which shows that the hyperbolicity condition can be relaxed.

Theorem 1: The network (4) has oscillatory solutions if it satisfies the following inequality

$$R \triangleq \frac{pB(1 - Bx_0)}{\kappa_0} > 1, \quad B \triangleq \prod_{m=1}^M b_m \quad (18)$$

where x_0 is the unique positive solution to $1/(1 + x_0^p) = Bx_0$, and κ_0 is determined by

$$\kappa_0 = \prod_{m=1}^M \sqrt{\mu^2 + b_m^2}, \quad \mu \triangleq \min_{0 < w < \infty} w \quad \text{s.t.} \quad \sum_{m=1}^M \arctan(w/b_m) = \pi \quad (19)$$

Proof: From Lemma 1, the proof of Y-oscillation is decomposed into three steps.

Step I – Satisfaction of condition (a): As has been shown in Sec. II-C, (4) has only one equilibrium $X_M^* = [x_0 \ x_0 \ \dots \ x_0]^T$ with $x_0 > 0$ determined by $f(x_0) = Bx_0$.

Step II – Satisfaction of condition (b): (b) can be proved following the derivations in [26].

Step III – Satisfaction of condition (c): The Jacobian matrix having at least one eigenvalue with positive real part is equivalent to a strictly unstable linearized system of (4) around the equilibrium, i.e., (16). So next we prove the strict instability of (16). The dynamics of (16) is characterized by its diagonal elements $\delta_j(s)$ ($1 \leq j \leq N$) in (17). For $\delta_1(s)$ we have $v_1 = 0$. Since both the amplitude and argument of $\prod_{m=1}^M (s + b_m)$ increase monotonically with the frequency on $[0, \infty)$, from graphic analysis [11] we know $\delta_1(s)$ is unstable if and only if $-\sigma$ (defined in (12)) is on the left of the intersection of $\prod_{m=1}^M (jw + b_m)$ and the negative real axis when w increases from 0 to ∞ , i.e., $-\kappa_0$ in (19) (cf. Fig. 3). So to have an unstable $\delta_1(s)$ we need:

$$\sigma > \kappa_0 \quad (20)$$

Similarly, we have $\delta_j(s)$ ($j = 2, 3, \dots, N$) is strictly unstable if and only if $-\sigma$ is on the left of the intersection of $(jw + b_k + v_j) \prod_{m=1, m \neq k}^M (jw + b_m)$ and the negative real axis when w increases from 0 to ∞ . Given that this intersection is on the left of $-\kappa_0$, we know that $G(s)$ is unstable if and only if (20) holds. Substituting σ in (12) into (20), we have $G(s)$ is strictly unstable if and only if (18) in Theorem 1 holds, i.e., condition (c) holds if (18) is satisfied. ■

Next we study the condition for stable synchronized oscillations, which is defined as:

Definition 2: (4) is synchronized if $\lim_{t \rightarrow +\infty} |x_{M,i}(t) - x_{M,j}(t)| = 0$ holds for any $1 \leq i, j \leq N$.

Remark 4: Only $x_{M,i}$ is used in the definition of synchronization. This is because according to the modeling assumption, $x_{M,i}$ corresponds to the concentration of inhibitor or enzyme, which can be regarded as the output of an oscillator. Under this definition, when the system is synchronized, $x_{m,i}$ ($1 \leq i \leq N$) may be identical or non-identical for $m \neq M$.

Theorem 2: The oscillator network (4) has stable synchronized oscillations only if

$$Nz_0/(N-1) < \sqrt{\mu_2^2 + (b_k + v_2)^2} \prod_{m=1, m \neq k}^M \sqrt{\mu^2 + b_m^2}, \quad z_0 = \max_{x>0} \frac{px^{p-1}}{(1+x^p)^2} \quad (21)$$

is satisfied, where v_2 is the second smallest eigenvalue of A and μ_2 is the minimal positive solution to $\arctan(\mu_2/(b_k + v_2)) + \sum_{m=1, m \neq k}^M \arctan \frac{\mu_2}{b_m} = \pi$.

Proof: A necessary condition for the synchronization in Definition 2 is the stability of synchronization manifold $x_{M,1}(t) = x_{M,2}(t) = \dots = x_{M,N}(t)$. So we check $y_{m,i} \triangleq x_{m,i} - \frac{\sum_{j=1}^N x_{m,j}}{N}$, which measures the deviation of the i th oscillator from the synchronization manifold. If the dynamics of $y_{m,i}$ is stable for all $1 \leq i \leq N$, then the synchronization manifold is stable.

From the definition of $y_{m,i}$ and (4), we can get the dynamics of $y_{m,i}$:

$$\begin{cases} dy_{1,i}/dt = \bar{h}_i - b_1 y_{1,i}, & \bar{h}_i = f(x_{M,i}) - \sum_{j=1}^N f(x_{M,j})/N \\ dy_{m,i}/dt = y_{m-1,i} - b_m y_{m,i}, & 2 \leq m \leq M, m \neq k \\ dy_{k,i}/dt = y_{k-1,i} - b_k y_{k,i} - \sum_{j=1, j \neq i}^N a_{i,j}(y_{k,i} - y_{k,j}) \end{cases} \quad (22)$$

Linearizing the system along the synchronization manifold yields:

$$\begin{cases} dY_1/dt = KY_M - b_1 Y_1 \\ dY_m/dt = Y_{m-1} - b_m Y_m, & 2 \leq m \leq M, m \neq k, \\ dY_k/dt = Y_{k-1} - b_k Y_k - AY_k \end{cases} \quad Y_m = \begin{bmatrix} y_{m,1} \\ y_{m,2} \\ \vdots \\ y_{m,N} \end{bmatrix} \in \mathbb{R}^{N \times 1} \quad (23)$$

In (23), matrix A is given in (5) and K is a matrix with diagonal elements given by $-z$ and off-diagonal elements given by $\frac{z}{(N-1)}$ where $z = \frac{px^{p-1}}{(1+x^p)^2}$.

Eqn (23) can be described in the frequency domain as shown in Fig. 2, where $H(s)$ is the same as (13) but L is replaced by $L = K$. The transfer function of (23) is $Q(s) = (I - H(s)K)^{-1}H(s)$. It can be verified that A and K commute, so we can diagonalize them simultaneously [24] and, thus diagonalize $Q(s)$ as $Q(s) = P \text{diag}(q_1(s), q_2(s), \dots, q_M(s)) P^{-1}$ with

$$q_i(s) = \frac{1}{(s + b_k + v_i) \prod_{m=1, m \neq k}^M (s + b_m) + \chi_i}, \quad i = 1, 2, \dots, N \quad (24)$$

where $\chi_1 = 0$ and $\chi_2 = \chi_3 = \dots = \chi_N = \frac{-N}{(N-1)}z = \frac{-N}{(N-1)} \frac{px^{p-1}}{(1+x^p)^2}$ are the eigenvalues of K . Note that they are different at different positions on the synchronization manifold.

Note $v_0 = 0$, $q_1(s)$ is stable, so we only consider $q_i(s)$ for $i = 2, 3, \dots, N$. Following Theorem 1, we know that $q_i(s)$ is stable if and only if χ_i resides on the right hand side of the intersection (denote it as $-\kappa_i$) of $q_i(s)$ with the negative real axis, which is determined by

$$\kappa_i = \sqrt{\mu_i^2 + (b_k + v_i)^2} \prod_{m=1, m \neq k}^M \sqrt{\mu_m^2 + b_m^2} \quad (25)$$

where μ_i is the minimal positive solution to $\arctan \frac{\mu_i}{b_k + v_i} + \sum_{m=1, m \neq k}^M \arctan \frac{\mu_i}{b_m} = \pi$. It can be verified that κ_i increases with v_i . So if $\chi_i > -\kappa_i$ holds for $i = 2$, which corresponds to the smallest v_i among $i = 2, 3, \dots, N$, then the synchronization manifold is stable. Given that χ_i is a function of x , (21) can be obtained by setting χ_2 to its minimal value among all $x > 0$. ■

Remark 5: Compared with the sufficient condition in [12], Theorem 2 is a necessary condition for global synchronization. In Sec. V we use simulations to estimate its conservativeness.

B. Biological insight

It can be verified that for $M \geq 2$, R in (18) increases with M , the length of the cyclic feedback. Given that a larger R makes (18) easier to satisfy, a longer cyclic feedback loop (i.e., a larger M , meaning involving more serial reactions) makes oscillation easier. Moreover, recalling the positive correlation between the averaged value of distributed delay and the length of feedback loop (cf. Remark 2), we can infer that a larger delay also makes oscillation easier.

From (21), we can see that with an increase in z_0 , a larger v_2 (i.e., a stronger intercellular interaction) is required to achieve synchronization. Given that for $p > 1$, z_0 can be verified an increasing function of the Hill coefficient p , we know that a system having a higher Hill coefficient (i.e., a higher cooperativity of end product repression) requires stronger coupling to maintain synchronization. Furthermore, we can also verify that a longer feedback chain makes the right hand side of the inequality in (21) lower, and thus makes (21) harder to satisfy. Given the positive correlation between the feedback loop length and the averaged distributed delay (cf. Remark 2), we can infer that a larger delay makes synchronization more difficult to maintain.

IV. OSCILLATION PERIOD ESTIMATION BASED ON MULTIVARIABLE HARMONIC BALANCE

A. Oscillation analysis based on harmonic balance technique

We reformulate the problem of oscillation analysis using a multivariable harmonic balance technique. This is motivated by the observation that $H(s)$ is a low pass filter thus higher order harmonics of oscillations in the closed-loop system can be safely neglected. Hence $x_{M,i}$ can be approximated by its zero-order and first-order harmonic components [16], [27]:

$$x_{M,i} = \alpha_i + \beta_i \sin(\omega t + \phi_i), \quad i = 1, 2, \dots, N \quad (26)$$

where α_i and β_i denote the amplitudes of the zero-order and the first-order harmonic components, respectively, and ω and ϕ_i denote the oscillation frequency and phase, respectively.

Since $f(\bullet)$ is a static nonlinear function, it can be approximated by describing functions [27]:

$$f(x_{M,i}) \approx \xi_i \alpha_i + \eta_i \beta_i \sin(\omega t + \phi_i) \quad (27)$$

$$\xi_i = \frac{1}{2\pi\alpha_i} \int_{-\pi}^{\pi} f(\alpha_i + \beta_i \sin(t)) dt, \quad \eta_i = \frac{1}{\pi\beta_i} \int_{-\pi}^{\pi} f(\alpha_i + \beta_i \sin(t)) \sin(t) dt \quad (28)$$

The describing function ξ_i is the gain of $f(\bullet)$ when the input is a constant α_i and the output is approximated by the zero-order harmonic component. The describing function η_i is the gain of $f(\bullet)$ when the input is a sinusoid of amplitude β_i and the output is approximated by the first-order harmonic component [27].

Consequently, α_i and β_i are expected to satisfy [16]:

$$(I - H(0)\Xi)\vec{\alpha} = 0, \quad (I - H(jw)\Pi)\vec{\beta} = 0 \quad (29)$$

where $\Xi = \text{diag}\{\xi_1, \dots, \xi_N\} \in \mathbb{R}^{N \times N}$, $\Pi = \text{diag}\{\eta_1, \dots, \eta_N\} \in \mathbb{R}^{N \times N}$, and

$$\vec{\alpha} = \begin{bmatrix} \alpha_1 & \alpha_2 & \dots & \alpha_N \end{bmatrix} \in \mathbb{R}^{N \times 1}, \quad \vec{\beta} = \begin{bmatrix} \beta_1 e^{j\phi_1} & \beta_2 e^{j\phi_2} & \dots & \beta_N e^{j\phi_N} \end{bmatrix} \in \mathbb{R}^{N \times 1}.$$

Note that (29) are referred to as harmonic balance equations.

Let Ξ^* and Π^* be matrices satisfying (29). Define two linear systems $G_0(s)$ and $G_1(s)$ as

$$G_0(s) \triangleq (I - H(s)\Xi^*)^{-1}H(s), \quad G_1(s) \triangleq (I - H(s)\Pi^*)^{-1}H(s) \quad (30)$$

$G_0(s)$ and $G_1(s)$ are obtained by replacing the nonlinearity $f(\bullet)$ with the constant gain computed from the describing functions. To ensure that the predicted oscillation frequency is biologically significant, oscillations at the estimated frequency must be stable, which, according to [28], can be ensured if both $G_0(s)$ and $G_1(s)$ are marginally stable (the only unstable poles are $s = 0$ and $s = \pm jw$, respectively). Therefore, estimating the collective period can be reduced to:

Problem 1: For the given $H(s)$ and nonlinear function $f(x)$ in (3), find w such that ① equation (29) is satisfied, and ② $G_0(s)$ and $G_1(s)$ in (30) are marginally stable.

B. Oscillation period of coupled negative cyclic feedback oscillators

Eqn (29) is very difficult to solve since in general Ξ and Π depend on $\vec{\alpha}$ and $\vec{\beta}$. Keeping in mind that we are interested in the collective period, we concentrate on solutions that describe synchronized oscillations. According to Definition 2, synchrony means that $x_{M,i}$ are identical, i.e., 1) the phases ϕ_i are identical; 2) the amplitudes α_i and β_i are respectively identical. Given that ξ_i and η_i are determined by α_i and β_i , we further have the equality of all ξ_i and all η_i :

$$\vec{\alpha} = \alpha \vec{1}, \quad \vec{\beta} = \beta \vec{1}, \quad \Xi = \xi I, \quad \Pi = \eta I, \quad \vec{1} \triangleq [1 \ 1 \ \dots \ 1]^T \in \mathbb{R}^{N \times 1}, \quad I = \text{diag}\{1, \dots, 1\} \in \mathbb{R}^{N \times N} \quad (31)$$

where α , β , ξ , and η are constants. Hence (29) reduces to

$$\left(\frac{1}{\xi}I - H(0)\right)\alpha \vec{1} = 0, \quad \left(\frac{1}{\eta}I - H(jw)\right)\beta \vec{1} = 0 \quad (32)$$

which further means that $\frac{1}{\xi}$ and $\frac{1}{\eta}$ are the respective eigenvalues of $H(0)$ and $H(jw)$ corresponding to the eigenvector with identical elements.

From (15), we know the eigenvalues of $H(0)$ are $\lambda_j = \frac{1}{(b_k + v_j)(\prod_{m=1, m \neq k}^M b_m)}$ for $j = 1, 2, \dots, N$. Since only λ_1 corresponds to eigenvectors with identical elements, we have (note $v_1 = 0$)

$$\xi = 1/\lambda_1 = \prod_{m=1}^M b_m \quad (33)$$

Similarly, we can get that the eigenvalues of $H(jw)$ are $\lambda_j(jw) = \frac{1}{(jw + b_k + v_j) \prod_{m=1, m \neq k}^M (jw + b_m)}$ for $j = 1, 2, \dots, N$. Since only λ_1 corresponds to eigenvectors with identical elements, we have

$$\eta = 1/\lambda_1(jw) = \prod_{m=1}^M (jw + b_m) \quad (34)$$

According to (28), η is real, thus the right hand side of (34) must be real. Given that μ is the minimal frequency that makes $\prod_{m=1}^M (jw + b_m)$ have zero imaginary part (angular π), the collective frequency is determined by μ in (19) and

$$\eta = \prod_{m=1}^M (j\mu + b_m) = - \prod_{m=1}^M \sqrt{\mu^2 + b_m^2} \quad (35)$$

To solve Problem 1, it remains to prove that $G_0(s)$ and $G_1(s)$ in (30) are marginally stable [28], or in other words: (1) $G_0(s)$ has one pole of $s = 0$ and the rest in the open left half plane and, (2) $G_1(s)$ has imaginary poles $s = \pm jw$ and the rest in the open left half plane.

Substituting Ξ and Π in (31) into (30) yields

$$G_0(s) = (I - \xi H(s))^{-1} H(s), \quad G_1(s) = (I - \eta H(s))^{-1} H(s) \quad (36)$$

with ξ and η given in (33) and (35), respectively.

First consider $G_0(s)$. From (16)-(17), we know that the eigenvalues of $G_0(s)$ are given by

$$\delta_j(s) = \frac{1}{(s + b_k + v_j) \prod_{m=1, m \neq k}^M (s + b_m) - \xi}, \quad j = 1, 2, \dots, N \quad (37)$$

Substituting ξ in (33) into (37), we know that the poles of $G_0(s)$ in (36) are the roots of

$$(s + b_k + v_j) \prod_{m=1, m \neq k}^M (s + b_m) - \prod_{m=1}^M b_m = 0, \quad j = 1, 2, \dots, N \quad (38)$$

For $j = 1$, since $v_1 = 0$, (38) has one root $s = 0$. It can also be derived that all the rest of the roots have negative real parts since for all s with a positive real part, the modulus of $\prod_{m=1}^M b_m$ is less than $\prod_{m=1}^M (s + b_m)$, which makes equality in (38) impossible. Similarly, we can get that for $j \neq 1$, all roots of (38) have negative real parts. Hence $G_0(s)$ is marginally stable.

Following the same line of reasoning, we can prove that the eigenvalues of $G_1(s)$ are

$$\delta_j(s) = \frac{1}{(s + b_k + v_j) \prod_{m=1}^M (s + b_m) - \eta}, \quad j = 1, 2, \dots, N$$

with η given in (35). And hence its poles are determined by the roots of

$$(s + b_k + v_j) \prod_{m=1, m \neq k}^M (s + b_m) + \prod_{m=1}^M \sqrt{\mu^2 + b_m^2} = 0, \quad j = 1, 2, \dots, N \quad (39)$$

where μ is given in (19).

For $j = 1$, we can verify that $s = \pm jw$ are roots of (39). We can also verify that all the other roots of (39) are stable, since for all s with a positive real part, the intersection of $\prod_{m=1}^M (s + b_m)$ and the negative real axis is less than $-\kappa_0 = -\prod_{m=1}^M \sqrt{\mu^2 + b_m^2}$, which makes the equality in (39) impossible. Similarly, for $j \neq 1$, we can derive that all roots of (39) are in the open left half plane. So $G_1(s)$ is marginally stable. Hence oscillations at the derived frequency μ are stable.

Proposition 1: The solution for the oscillation frequency w in Problem 1 is given by $w = \mu$ where μ is defined in (19).

From the above derivation, we can see that the collective oscillation period is expected to be

$$T_{\text{collective}} = 2\pi/\mu \quad (40)$$

C. Biological insight

The collective period in (40) is given in terms of the dimensionless parameters in (2). The actual collective frequency in dimensional parameters are given by $\Omega = \varsigma\mu$ where μ is the minimal positive solution to $\sum_{m=1}^M \arctan \frac{\mu}{b_m} = \sum_{m=1}^M \arctan \frac{\mu}{k_m/\varsigma} = \pi$. So the actual collective frequency is the minimal positive solution to $\sum_{m=1}^M \arctan \frac{\Omega}{k_m} = \pi$. This means that the collective frequency Ω increases with an increase in the degradation rate of each component (k_m), but it is independent of the rates of transcription, translation, and synthesis. These give insights into the basic determination mechanism of the collective period in coupled biological oscillators, and may further provide guidance in synthetic biology design.

From the above derivation, we can see that under interaction (3), the collective period is only determined by k_m ($m = 1, 2, \dots, M$), and it is independent of intercellular coupling. The results are obtained based on analytical treatment of a network of coupled gene regulatory oscillators and they corroborate the results in [15], which are obtained using the phenomenological single-variable phase model and state that the strength of intercellular coupling does not affect the

collective period of circadian rhythm oscillator networks. In fact, this is reasonable since the coupling is similar to the linear consensus protocol [29], which only affects the process to synchronization. Moreover, when the degradation rate is fixed, it can be inferred that Ω decreases with an increase in the length of the feedback loop M . Therefore, a longer feedback loop corresponds to a longer collective period. Furthermore, recall that the effect of distributed delay amounts to increasing the length of the feedback loop and the increased length is proportional to the averaged delay, hence, a larger delay in individual loops means a longer collective period.

Remark 6: If the coupling is different from (3), it may affect the collective period, as exemplified by the mutual repressive coupling in [18].

V. NUMERICAL STUDY

We considered a network of nine oscillators coupled via the second reactant. The coupling strengths $a_{i,j}$ were chosen from a uniform distribution on $[0, 20]$ and the coupling topology is verified to be connected. First we tested our oscillation condition, with results given in Table I. It can be seen that oscillation can be obtained only when the parameters satisfy $R > 1$ in (18).

TABLE I
TEST OF THE OSCILLATION CONDITION

p	b_1	b_2	b_3	b_4	b_5	b_6	b_7	b_8	b_9	R	Simulation results
3	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1.6898	Oscillation
3	0.5	0.6	0.7	0.8	0.9	0.8	0.7	0.6	0.5	1.5733	Oscillation
3	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	1.5571	Oscillation
3	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.3549	Oscillation
3	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	1.0707	Oscillation
3	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9819	No oscillation
3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.4721	No oscillation

We then compared our synchronization condition with the sufficient synchronization condition in [12]. The gap between the two conditions is shown in Table II. It is worth noting that extensive numerical simulations showed that our synchronization condition is minimally conservative because despite the fact that it is a necessary synchronization condition, it successfully ensured synchronization for all 10^6 runs with initial conditions randomly chosen from the interval $[0, 10^3]$.

We also verified the estimated collective periods in oscillatory cases. The results (in Table III) show that the estimated values approximate the actual collective periods closely.

TABLE II

COMPARISON OF THE REQUIRED NETWORK CONNECTIVITY v_2 TO ACHIEVE SYNCHRONIZATION

$b_1 = b_2 = \dots = b_9$	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85
The required v_2 in [12]	178.13	82.78	40.94	21.24	11.40	6.22	3.35	1.71
The required v_2 in this paper	127.98	59.45	29.36	15.19	8.11	4.37	2.30	1.09

TABLE III

COMPARISON BETWEEN THE ESTIMATED COLLECTIVE PERIOD [s] AND THE ACTUAL COLLECTIVE PERIOD [s]

$b_1 = b_2 = \dots = b_9$	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85
Actual value	40.9	36.2	32.3	29.0	26.2	23.9	22.03	20.4
Estimated value	36.0	32.7	30.0	27.7	25.7	24.0	22.5	21.1
Estimation error	-11.9%	-9.67%	-7.12%	-5.86%	-1.91%	0.42%	0.89%	3.4%

VI. CONCLUSIONS

Biological rhythms are generated by networks of interacting cellular oscillators. The mechanisms that describe how the collective oscillation patterns arise from autonomous cellular oscillations are poorly understood. Based on a network of coupled negative cyclic feedback oscillators, we studied the oscillation/synchronization condition and collective period of coupled biochemical oscillators by using a multivariable harmonic balance technique. We gave oscillation and synchronization conditions of coupled negative cyclic feedback oscillators. We also analytically estimated the collective oscillation period of the oscillator network and examined how it is affected by the parameters of biochemical reactions. The results are confirmed by numerical simulations and can provide guidance in synthetic oscillator design in biology.

REFERENCES

- [1] Y. Q. Wang, Y. Hori, S. Hara, and F. J. Doyle III. The collective oscillation period of inter-coupled goodwin oscillators. In *Proc. 51th IEEE Conf. Decision Control*, pages 1627–1632, Maui, USA, 2012.
- [2] O. V. Popovych and P. A. Tass. Macroscopic entrainment of periodically forced oscillatory ensembles. *Prog. Biophys. Mol. Biol.*, 105:98–108, 2011.
- [3] H Wünsche, S Bauer, J Kreissl, O Ushakov, N Korneyev, F Henneberger, E Wille, H Erzgräber, M Peil, W Elsässer, and I Fischer. Synchronization of delay-coupled oscillators: a study of semiconductor lasers. *Phys. Rev. Lett.*, 94:163901, 2005.
- [4] L. Herrgen, S. Ares, L. G. Morelli, C. Schröter, F. Jülicher, and A. C. Oates. Intercellular coupling regulates the period of the segmentation clock. *Curr. Biol.*, 20:1244–1253, 2010.
- [5] B. Novák and J. J. Tyson. Design principles of biochemical oscillators. *Nat. Rev. Mol. Cell Biol.*, 9:981–991, 2008.
- [6] C. Fall, E. Marland, J. Wagner, and J. J. Tyson, editors. *Computational Cell Biology*. Springer, New York, 2005.

- [7] N. Stephanopoulos, A. Aristidou, and J. Nielsen, editors. *Metabolic engineering principles and methodologies*. Academic Press, San Diego, 1998.
- [8] J. Griffith. Mathematics of cellular control processes: negative feedback to one gene. *J. Theoret. Biol.*, 20:202–208, 1968.
- [9] A. Hunding. Limit-cycles in enzyme-systems with nonlinear negative feedback. *Biophys Struct Mech.*, 1:47–54, 1974.
- [10] J. Tyson. On the existence of oscillatory solutions in negative feedback cellular control processes. *J. Math. Biol.*, 1:311–315, 1975.
- [11] Y. Hori, T. Kim, and S. Hara. Existence criteria of periodic oscillations in cyclic gene regulatory networks. *Automatica*, 47:1203–1209, 2011.
- [12] A. Hamadeh, G. Stan, R. Sepulchre, and J. Goncalves. Global state synchronization in networks of cyclic feedback systems. *IEEE Trans. Autom. Control*, 57:478–483, 2012.
- [13] P. Papp. Analysis of biochemical phase shift oscillators by a harmonic balancing technique. *Math Biol*, 25:203–224, 1976.
- [14] Y. Hori, M. Takada, and S. Hara. Biochemical oscillations in delayed negative cyclic feedback: Existence and profiles. *Automatica*, 49:2581–2590, 2013.
- [15] C. Liu, D. Weaver, S. H. Strogatz, and S. M. Reppert. Cellular construction of a circadian clock: period determination in the Suprachiasmatic Nuclei. *Cell*, 91:855–860, 1997.
- [16] T. Iwasaki. Multivariable harmonic balance for central pattern generators. *Automatica*, 44:3061–3069, 2008.
- [17] A. Turing. The chemical basis of morphogenesis. *Phil. Trans. R. Soc. B*, 237:37–72, 1952.
- [18] Y. Q. Wang, Y. Hori, S. Hara, and F. J. Doyle III. Intercellular delay regulates the collective period of repressively coupled gene regulatory oscillator networks. *IEEE Trans. Autom. Control*, 59:211–216, 2014.
- [19] N. MacDonald. *Time lags in biological models*. Springer, Berlin, 1978.
- [20] J. Tyson and H. Othmer. The dynamics of feedback control circuits in biochemical pathways. *Prog. Theo. Biol.*, 5:1–62, 1978.
- [21] J. J. Tyson. Periodic enzyme synthesis reconsideration of the theory of oscillatory repression. *J. Theor. Biol.*, 80:27–38, 1979.
- [22] N. Bagheri, S. Taylor, K. Meeker, L. Petzold, and F. J. Doyle III. Synchrony and entrainment properties of robust circadian oscillators. *J. R. Soc. Interface*, 5:S17–S28, 2008.
- [23] T. To, M. A. Henson, E. Herzog, and F. J. Doyle. A molecular model for intercellular synchronization in the mammalian circadian clock. *Biophys. J.*, 92:3792–3803, 2007.
- [24] R. Horn and C. Johnson. *Matrix analysis*. Cambridge University Press, London, 1985.
- [25] A. Pogromsky, T. Glad, and H. Nijmeijer. On diffusion driven oscillations in coupled dynamical systems. *Int. J. Bifurcation Chaos*, 9:629–644, 1999.
- [26] H. El Samad, D. Del Vecchio, and M. Khammash. Repressilators and promotilators: loop dynamics in synthetic gene networks. In *Proc. 2005 American Contr. Conf.*, pages 4405–4410, Portland, USA, 2005.
- [27] H. K. Khalil. *Nonlinear systems*. Prentice Hall, New Jersey, 2002.
- [28] T. Glad and L. Ljung. *Control theory - multivariable and nonlinear methods*. Taylor & Francis, 2000.
- [29] R. Olfati-Saber, J. A. Fax, and R. M. Murray. Consensus and cooperation in networked multi-agent systems. *Proc. IEEE*, 95:215–233, 2007.